

06/06/21

Hydrogen removed  
as proton.

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### DNA ligase

- very well known.
- also called molecular sutures / Molecular glue.
- essential in
  - DNA replication
  - DNA repair
- DNA ligase plays very significant role in RDP

Working (From Linheger)

### of Ecoli.

- DNA ligase which are available to perform ligation reaction under *in vitro* condition are of two different types

- (i) E-coli DNA ligase → It needs NAD<sup>+</sup> as co-factor
- (ii) T4 DNA ligase → It needs ATP as co-factor

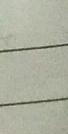
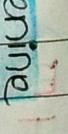
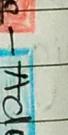
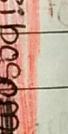
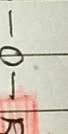
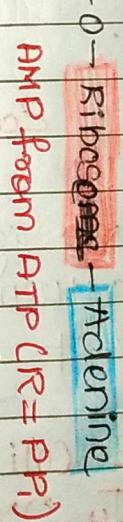
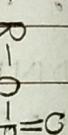
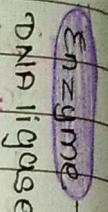
- DNA ligase is initially inactive
- ATP + NADP<sup>+</sup> required

### # Mode of action of DNA ligase is as follows.

- In each of the 3 steps one phosphodiester bond is formed at the expense of another
- Step ① & ② lead to activation of the 5' phosphate in the nick.

**① Adenylylation of DNA ligase.**

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Enzyme

-NH<sub>2</sub>-P-O-Ribose

-Adenine

NMN

(from NAD $^+$ )

PP<sub>i</sub>

(from ATP)

OR

AMP from ATP ( $\text{R} = \text{PP}_i$ )

or NAD $^+$  ( $\text{R} = \text{NMN}$ )

**③ Displacement of AMP seals nick**

②

Activation of  
5' phosphate in  
nick

Enzyme-AMP

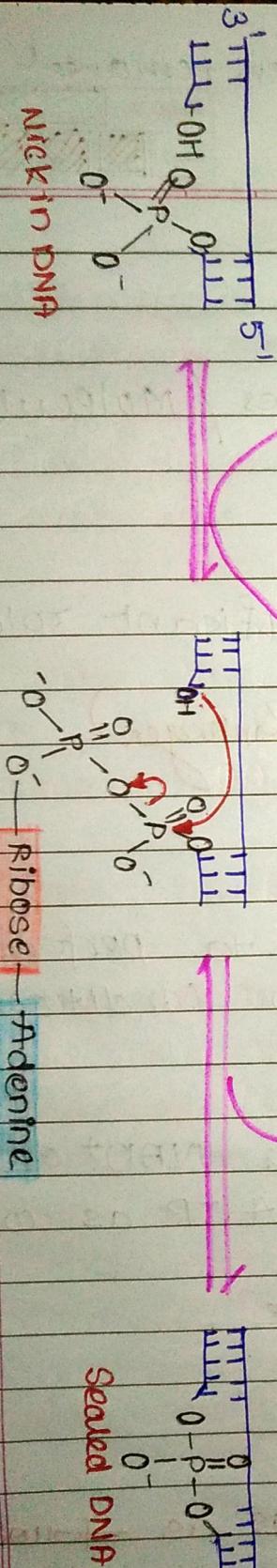
Enzyme-NH<sub>3</sub><sup>+</sup>

-O-P-O-Ribose-Adenine

AMP

Sealed DNA

NICK IN DNA



• E. coli DNA ligase = NAD<sup>+</sup> (very common).

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- An AMP group is transferred first to a Lys residue on the enzyme & then to the 5' phosphate in the nick.
- In step ③, the 3'-hydroxyl group attacks this phosphate & displaces AMP, producing a phosphodiester bond to seal the nick.
- In the E-coli DNA ligase reaction AMP is derived from NAD<sup>+</sup>.
- The DNA ligases isolated from a number of viral & eukaryotic sources use ATP rather than NAD<sup>+</sup> & they replace phosphate rather than Nicotinamid mononucleotide (AMN) in step ①

→ BMHAI

### o DNA Polymerase

- i) The most important enzyme of DNA replication and recombination
- ii) In prokaryotes, the five different types of DNA polymerase are found.

for eg:- I, II, III, IV, V

iii) Among them Pol I, II, III are commonly used by the cell.

- In prokaryotes there are 5 different DNA Pol.
- In Eukaryotes there are 13 different DNA Pol.

### Prokaryotic DNA Pol.

Activities	DNA Pol I (300-400)	DNA Pol II (30-40)	DNA Pol III (~10)
5' → 3' Polymerization	Yes	Yes	Yes
5' → 3' exonuclease	Yes	Yes	Yes
5' → 3' exonuclease	Yes	No	No
Structure	Single polypeptide chain	Single polypeptide chain	Multimeric complex
Discoverer	Arthur Kornberg (1956)	Thomas Kornberg (1970)	Thomas Kornberg & Malcolm Gofman (1970)

## Eukaryotic DNA Pol.

1)  $\alpha$

2)  $\beta$

3)  $\gamma$

4)  $\delta$

5)  $\epsilon$  (main ECP)

6)  $\zeta$

7)  $\theta$

8)  $\lambda$

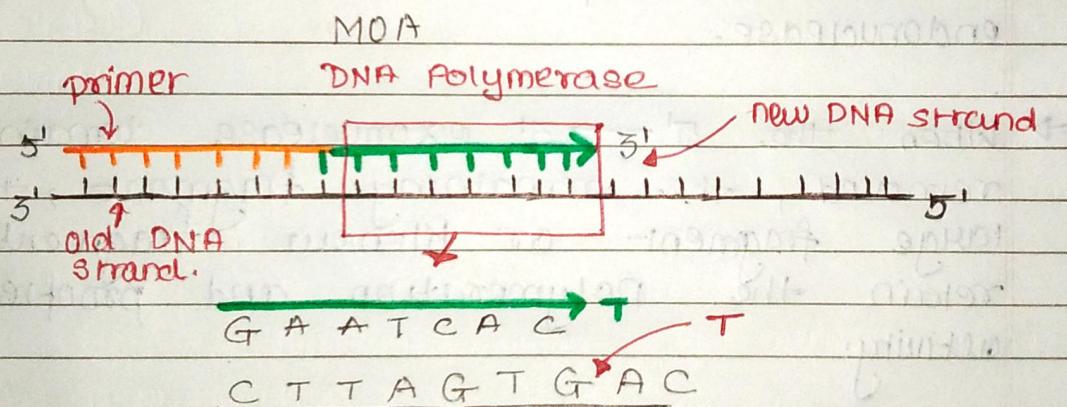
9)  $\kappa$

10)  $\lambda$

11)  $\mu$

12) Rev-1

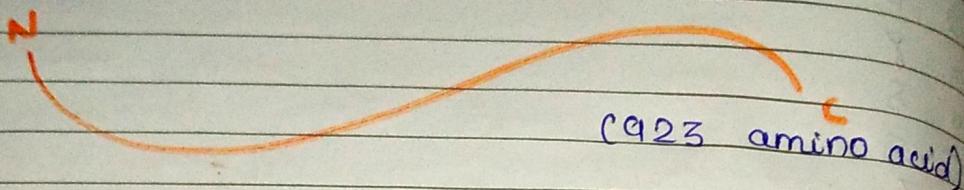
13)  $\eta$



## \* DNA Pol I

⇒ The search for an enzyme (DNA Pol I) that could synthesize DNA discovered in 1956 by Arthur Kornberg.

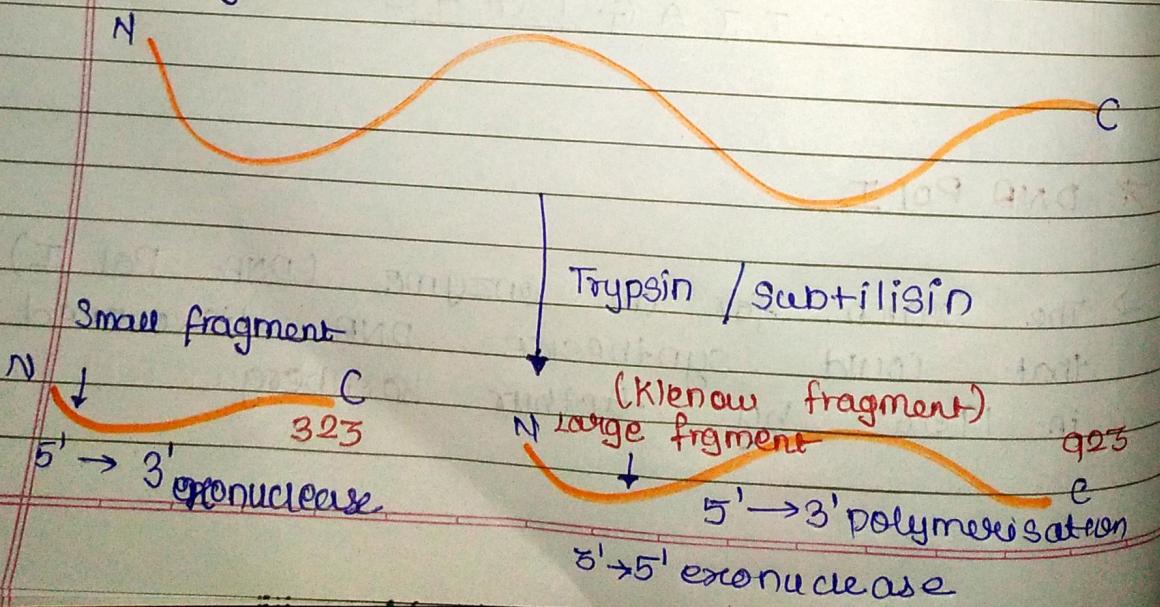
⇒ The molecular mass of DNA Pol I is 109 kDa.



⇒ DNA polymerase I, instead it performs host of clean-up functions during replication, recombination & repair.

⇒ The polymerase's special function are enhanced by its  $5' \rightarrow 3'$  exonuclease activity and distinct from the  $3' \rightarrow 5'$  proofreading endonuclease.

⇒ When the  $5' \rightarrow 3'$  exonuclease domain is removed, the remaining fragment, the large fragment or Klenow fragment, retain the polymerization and proofreading activity.



⇒ Klenow fragment possesses good processivity as compared to DNA Pol I.

### # Application

- 1 Helps in cDNA preparation
- 2 Exclusive used in random primer method
- 3 Nick translation
- 4 Used in Sanger method
- 5 Helpful to generate blunt end (end filling)
- 6 Effective in End labelling.